and/or

(f) the ribonucleic acids or peptides effecting alteration of the preselected cellular function are used directly for isolation and identification of a ligand molecule to said ribonucleic acids or peptides.--

R E M A R K S

With respect to the claims listed as pending, the Examiner should note that claims 34-36 were cancelled in the Amendment filed July 29, 1999.

The Examiner states claim 38 depends from claim 19 which has been cancelled. Claim 38 has been amended to depend from claim 1.

Claim Objections

The Examiner objects to claim 35. Claim 35 has been cancelled as noted above.

Claim Rejections - 35 U.S.C. §112

Claims 9, 20, 21, 34, 36 and 37 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. This rejection is moot as to claims 34 and 36 which have previously been cancelled. The rejection of claims 9, 20, 21 and 37 has been overcome as discussed below.

Claim 20 now recites the term "cellular function" as suggested by the Examiner.

Claims 9 and 37 have been amended to recite proper Markush language.

Claim 21 has been amended to delete the term "these" and set forth proper antecedent basis.

Claim Rejections - 35 U.S.C. §103

Claims 1-6, 8-18, 20-24, 30, 31, 34-42, 48, 53 and 59-68 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kay et al (U.S. Patent No. 5,747,334) in view of Woodring et al (sic), Lam et al (U.S. Patent No. 5,650,489), Huse (U.S. Patent No. 5,770,434), Burke et al (U.S. Patent No. 4,618,578) and Wong et al. This rejection is respectfully traversed.

Amendments To Independent Claim 1

Initially, applicants will comment on the amendment to independent claim 1 and new independent claim 69. The amendment of claim 1 is supported in the specification on page 3, lines 34-38. The limitation has been introduced in order to clearly distinguish an "altered cellular function" from simple emergence in a cell of an expression product from a library.

New Independent Claim 69

Independent claim 69 uses the wording "phenotypic trait" as related to changes in biological function of the cell. In step c of claim 69, the screening method has been defined by means of a definition of the "preselected phenotypic trait" which specifies that "... the preselected phenotypic trait has been so selected that an observed alteration thereof in a transduced cell provides an indication that said ribonucleic acid(s) or peptide(s) expressed in the transduced cell affects biological function(s) of the transduced cell, where said biological function(s) participate in generating the preselected phenotypic trait ...."

In applicant's opinion, this wording of claim 69 very accurately defines the distinction between the method of the

invention and traditional ligand capture methods such as the method of Kay et al. In the traditional methods, the screening step is in no way chosen to provide an indication that the expression product from a transduced member of a random oligonucleotide library has affected biological functions in the transduced cell; on the contrary, the traditional methods using a known capture ligand cannot distinguish whether, e.g., a captured peptide has affected any functions in the cell which is different from the effects exerted by non-captured expression products.

In contrast, the present method relies completely on such a choice of screening method, since the object of the invention is to identify peptides or RNA which can effect in vivo changes of cellular mechanisms and thereby aid in detecting hitherto unknown biomolecules inside the cells or hitherto unknown functions of known biomolecules.

Support for the wording of claim 69 is found in the specification on page 3, line 34, through page 4, line 2, together with the exemplified screening methods in Examples 2-4.

Kay et al

Referring now to the applied prior art, in the Examiner's discussion on pages 6-7 of features disclosed in Kay et al, it appears that the Examiner still finds that Kay et al discloses a method for identifying biologically active peptides or RNA.

Applicants wish to note that they have previously pointed out that the method of Kay et al provides no guarantee that a peptide identified according to the method described therein will be biologically active (this has to be confirmed in subsequent steps), whereas the present method will exclusively identify peptides and RNA which are active in a living cell, i.e., biologically active. For this reason alone, applicants submit that the independent claims on file are not rendered obvious over Kay et al combined with any of the cited secondary references.

Further, the use of eukaryotic transduction of the "one gene - one cell" type as claimed in the present independent claims is not taught or suggested by Kay et al. In fact, as pointed out previously, Kay et al specifically suggests that electroporation is used for introduction of nucleic vectors in eukaryotic host cells (cf. the paragraph bridging columns 28 and 29) - in eukaryotic cells traditional electroporation leads to introduction of several hundreds and up to thousands of vectors in one cell. Hence, Kay et al does not provide any guidance to the skilled person that a "one gene - one cell" transduction approach would be desirable let alone essential. On the contrary, Kay et al teaches away from this essential feature of the present invention.

Applicants note that the Examiner has withdrawn the citation of the reference by Sigmund C. Previously, that reference was used by the Examiner to demonstrate that Kay et al inherently relates to a one gene - one cell transduction method. Applicants submit that the withdrawal of the citation of the Sigmund C reference in essence means that the Examine acknowledges that Kay et al does in fact teach away from such an approach.

Finally, it appears that the Examiner is of the opinion that the method of Kay et al identifies peptides or RNA which alters a preselected cellular function. Applicants do not share this viewpoint. In applicants' opinion, it stretches the English language too far to interpret the wording "altering a preselected cellular function" to embrace the emergence of a specific binding peptide inside or on the surface of a cell. However, in order to completely distinguish the prior art methods like Kay et al with respect to this feature, applicants have amended claim 1 as indicated above by reciting language corresponding to the language on page 3, lines 34-38 in the specification.

Applicants have not made a similar amendment to claim 42 for the following reason: Claim 42 requires that the screening

detects up or down regulation of a preselected cellular function. In applicants' opinion, up and down regulation means that the cellular function already exists to some degree in the cells prior to the transduction and that the cells have themselves performed a regulation of the function in response to a "positive" transduction event. The method according to Kay et al, on the contrary, relies on the detection of a previously non-existing characteristic of the cell in question.

Wright et al

The Examiner states on page 7 of the Office Action that Woodring et al (sic) (Wright et al) teaches "... the use of totally random synthetic nucleic acids to alter a cellular function in a cell by direct activity or by the ligand binding of the nucleic acid or its expressed RNA or peptide ...." Applicants do not agree with this interpretation of the teachings of Wright et al.

Applicants do agree that Wright et al relates to the identification outside cells of DNA sequences derived from a random library using a technology termed CASTing. However, Wright et al screens the random DNA fragments against purified preparations of myogenin or myotube nuclear extracts, enriches for DNA binding to these preparations and subsequently selects, clones and sequences the positive binders.

The method taught in Wright et al can be summarized as follows:

1. Preparation of double-stranded randomized DNA library.
2. Admixing the library with purified myogenin fusion protein or myotube nuclear extracts (which contains myogenin) thereby obtaining binding between certain members of the library and myogenin.
3. Addition of anti-myogenin coated magnetic beads, thereby obtaining non-covalent coupling of myogenin to the beads.

4. Retention of magnetic beads while washing with buffer, thereby removing the majority of the library members (except those bound to myogenin on the magnetic beads).
5. Resuspension of beads, denaturing of myogenin and anti-myogenin, and performing 9 cycles of PCR amplification of residual random fragments, thereby enriching for myogenin binding members of the library.
6. Withdrawing ¼ of the PCR product of step 5 and admixing with purified myogenin fusion protein or myotube nuclear extracts (which contains myogenin).
7. Repetition of steps 3 through 6 up to completion of 6 cycles CASTing.
8. Cloning and subsequent sequencing of DNA bound to beads.

Hence, it is impossible to find any teaching in Wright et al where a random library is used to isolate members which alter a cellular function in a cell during the identification steps, since all steps up to step 8 are performed using cellular extracts. Furthermore, the material identified according to Wright et al is DNA, not a peptide or RNA - in fact, there is no suggestion in Wright et al that products encoded by the isolated DNA should or could have any biological relevance at all since myogenin (which is used as capturing agent) is a DNA binding protein. Furthermore, the DNA fragments isolated according to Wright et al are not indicated as being coding sequences, and it is most unlikely that any of them encode a product which is biologically active. It should be noted that the cloning in step 8 only serves to render the isolation and sequencing of positive DNA fragments possible but that no expression of the DNA is effected.

Thus, Wright et al does not teach 1) the use of totally random nucleic acids to alter a cellular function or 2) the identification of biologically active RNA or peptides.

Huse

The Examiner states on page 7 of the Office Action that U.S. Patent No. 5,770,434 (Huse) teaches "... the use of synthetic nucleic acids coding for totally random peptide sequences which are expressed in host cells to identify those sequences which alter cellular function ...."

Again, applicants do not agree with the Examiner. Huse relates to a method for synthesizing and expressing oligonucleotides having a desirable bias of random non-degenerate codons. In other words, Huse relates to an alternative method for obtaining synthesis of random codons as those described in the present specification on page 10, lines 22 38.

It is briefly mentioned in Huse that such randomized codon sequences can be expressed in eukaryotic cells, cf. column 10, lines 40-41. However, no examples are given of the use of such randomized codon sequences in the screening for those which encode expression products which will alter a preselected cellular function as presently claimed. In fact, the only use of the randomized sequences which is set forth in Huse is in the identification of binding partners in phage display. That is, the method is in many aspects similar to that of Kay et al, i.e., it entails isolation of members from a library of random nucleic acids, said members encoding ligands to a known binding partner (cf., e.g., column 33, lines 53-56 of Huse).

Without any indications to the contrary, the teaching that the skilled artisan can extract from Huse is that the random codon sequences therein can be used for isolation/identification of ligands to a known binding partner.

Thus, Huse does not in any way teach a method where a screening is performed that isolates expression products which alter a preselected cellular function as presently claimed. Furthermore, with reference to new claim 69, the method of Huse does not relate to a method where the screening method employed

provides an indication that "positive" library members have affected biological functionality of the cell.

Therefore, since neither Kay et al, Wright et al, nor Huse teach a screen for cells which alters a preselected cellular function, the invention as claimed in claims 1, 42 and 69 is unobvious over the cited prior art taken alone or in combination.

Other Applied References

It should be noted that none of the other references cited produce either the feature of screening for cells which alters a preselected cellular function, or the concept of selecting a cellular function the alteration of which indicates that a biological function in the cell has been affected; neither does any of the other secondary references provide information which will overcome the teaching of Kay et al that electroporation is a suitable technology. Notably, U.S. Patent No. 5,650,489 (Lam et al) cited by the Examiner does not provide any of these features.

Claims 25 and 26 are rejected under 35 U.S.C. 103(a) as being unpatentable over the above applied prior art, and further in view of Stemmer et al. This rejection is respectfully traversed.

Stemmer et al

As disclosed in the last response, Stemmer et al is not prior art because the filing date of the Danish priority application antedates the §102 date and the PCT publication date of Stemmer et al. In this regard, it is again pointed out that the Danish priority application in the present case is an English language document and that the subject matter of claims 25 and 26 is clearly disclosed therein.

Allowable Claim 7

Claim 7 is objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims. Claim 7 has been rewritten in independent form as claim 70.

Summary

Using Kay et al as the primary reference must mean that the skilled person considers all relevant teachings therein, and these have to be applied when combining Kay et al with any secondary reference.

Compared with the presently claimed method, Kay et al fails to teach a number of essential features:

1) Kay et al does not teach a method where an in vivo biologically active peptide or RNA is necessarily identified, whereas the presently claimed method will only identify such peptides or RNA. None of the cited secondary references provides the necessary means or motivation to arrive to the presently claimed method when combined with Kay et al.

2) Kay et al does not teach a one gene - one cell transduction of eukaryotic cells. In fact, Kay et al teaches away from this essential feature since electroporation of eukaryotic cells is suggested and no other teachings are provided that gives the skilled person an incentive to use a one gene - one cell transduction approach. None of the cited secondary references provides any incentive to revert this teaching of Kay et al.

3) Kay et al does not teach a screening for altered cellular function or for altered phenotype caused by biological functions being affected by the expression products transduced into eukaryotic cells. As detailed above, neither Wright et al nor Huse (or any other cited secondary reference) teaches such a screening.

Since the remaining prior art rejections all relate to claims dependent on claims 1 and 42, and since these claims are not obvious over the prior art as demonstrated above, the remaining claim rejections cannot stand.

Further, the rejections of claims 25 and 26 cannot stand for the simple reason that Stemmer et al is not prior art.

Finally, claim 70 is prima facie in condition for allowance.

In view of the foregoing, it is believed that the present application is in condition for allowance. Accordingly, and especially in view of the fact that this is a response to the third action on the merits, early and favorable action is respectfully requested.

In the event the present response does not place the application in condition for allowance, it is respectfully requested that Examiner Sandals telephone the undersigned attorney so that a telephone conference in which Primary Examiner Brusca participates may be arranged before the issuance of a final rejection.

The Commissioner is hereby authorized to charge any fees due in connection with the present application to Deposit Account 06-1358.

Respectfully submitted,

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